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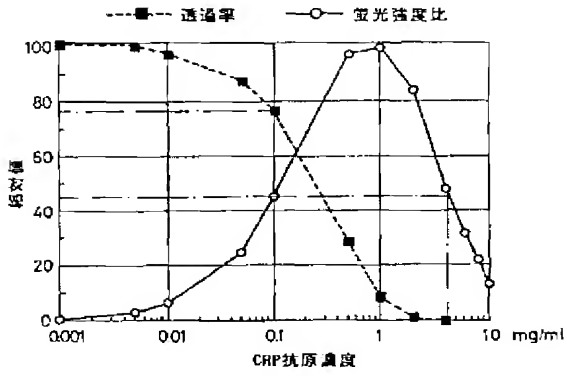
(54)【発明の名称】　リポソームを用いた免疫測定法

(57)【要約】　（修正有）
【構成】　リポソームを用いた免疫測定法において、既知の濃度のカルボキシフルオレセイン蛍光色素について予め蛍光強度比と透過率との検量線を作成しておき、未知検体についてカルボキシフルオレセイン蛍光色素の蛍光強度比と共に透過率も同時に測定し、得られた両測定値を前記検量線に当てはめることにより、未知検体中のカルボキシフルオレセイン蛍光色素濃度を決定する。
【効果】　短時間に確実にカルボキシフルオレセイン蛍光色素濃度を決定することができる。



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【特許請求の範囲】

【請求項１】　リポソーム内にカルボキシフルオレセイン蛍光色素を封入し、このリポソーム膜にタンパク抗原または特異抗体を共有結合させてリポソーム膜表面に抗原抗体複合体を形成させ、これに補体を作用させることにより膜攻撃複合体を形成し、該膜攻撃複合体によりリポソーム膜を破壊し、リポソーム内に封入されていたカルボキシフルオレセイン蛍光色素をリポソーム外へ放出させ、この放出されたカルボキシフルオレセイン蛍光色素の蛍光強度を測定することにより抗原量及び抗体量を測定することからなるリポソームを用いた免疫測定法において、カルボキシフルオレセイン蛍光色素の蛍光強度比と共に透過率も同時に測定し、得られた両測定値に基づいてカルボキシフルオレセイン蛍光色素の濃度を決定することを特徴とするリポソームを用いた免疫測定法。

【請求項２】　既知の濃度のカルボキシフルオレセイン蛍光色素について予め蛍光強度比と透過率との検量線を作成しておき、未知検体について測定された蛍光強度比及び透過率の測定値を前記検量線に当てはめることにより、未知検体中のカルボキシフルオレセイン蛍光色素濃度を決定することからなる請求項１の方法。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明はリポソームを用いた免疫測定法に関する。更に詳細には、本発明は蛍光体としてカルボキシフルオレセイン蛍光色素を使用するリポソームを用いた免疫測定法において、短時間に確実にカルボキシフルオレセイン蛍光色素濃度を決定することができるリポソーム免疫測定法に関する。

【０００２】

【従来の技術】抗原抗体反応による免疫測定法は、特異性及び感度の点で非常に優れているので、理化学研究，医学生物学研究及び臨床検査などの諸分野で広く使用されている。この抗原抗体反応を基にして、検体の微量定量を行うために、放射性同位元素によるラジオイムノアッセイ（ＲＩＡ），酵素によるエンザイムイムノアッセイ（ＥＩＡ）などの方法が開発されてきた。

【０００３】しかし、ＲＩＡは感度の点で非常に優れているが、標識物質として放射性同位元素を使用するので、第１種放射線取扱主任者の有資格者が必要であり、測定は特定の放射能管理施設内でなければ実施出来ず、更に、放射性同位元素の廃棄物処理のために特別な施設を必要とするなどの問題点がある。

【０００４】ＥＩＡでは標識物質として酵素を用いるため、ＲＩＡのような問題は生じないが、未反応の標識物質を分離しなければならないなどの問題点があり、測定には非常に手間がかかる。

【０００５】これらの方法の他に、最近、リポソームを使用する免疫測定法（ＬＩＬＡ）が提案された。このＬＩＬＡは従来のＲＩＡやＥＩＡと異なり、未反応標識物

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質の分離操作を必要としない均一系で、しかも、迅速に測定できるなどの利点がある。

【０００６】リポソームは、同一分子内に親水基と疎水基の両方を有する両親媒性の複合脂質（例えば、レシチン，コレステロール，ホスファチジン酸など）を一定の温度以上で緩衝液に懸濁することで調製される脂質二重層からなる小胞体である。このリポソーム膜に、二官能性架橋剤を用いてタンパク抗原又は特異抗体を共有結合する。リポソーム内に蛍光標識としてカルボキシフルオレセイン（ＣＦ）を封入する。ＣＦはフルオレセイン系の色素で、水溶性が高く、容易にリポソーム内に封入される。これに特異抗体又は抗原を反応させると、リポソーム膜表面に抗原抗体複合体が形成される。この時、補体が存在すると、補体が活性化され、膜攻撃複合体が形成される。それがリポソーム膜に結着して脂質二重層の透過性が上昇し、リポソーム内のＣＦが外部溶媒中に流出してくる。ＣＦは高濃度でリポソーム内に封入されている時は自己消光により蛍光を発しないが、リポソーム外に流出して外部溶媒に希釈されると蛍光を発するようになる。この蛍光強度を測定することにより抗原量及び抗体量を求めることができる。

【０００７】

【発明が解決しようとする課題】図３に示されるように、ＣＦはモル濃度の増加に従い、或る濃度（C_p）までは蛍光強度と濃度が比例関係を保つが、C_pを超える比較的高濃度の領域では、逆に蛍光濃度が低下する特性を示す。従って、蛍光強度$I_{f,A}$におけるＣＦの濃度はC_1とC_2の二種類の値が得られ、何方の濃度が正しいのか決定できなくなってしまう。

【０００８】このため、従来は、ＣＦの蛍光強度から濃度を推定する検査方法において、ＣＦの濃度領域を限定するか、若しくは、測定対象を幾通りかに希釈して、前後の特性から濃度を推定するなどの迂遠で冗長な方法が採られていた。

【０００９】従って、本発明の目的は、従来のような迂遠な方法を採ることなく、ＣＦの濃度を短時間に確実に推定できる新規な蛍光検出方法を提供することである。

【００１０】

【課題を解決するための手段】前記課題を解決するために、本発明は、リポソーム内にカルボキシフルオレセイン蛍光色素を封入し、このリポソーム膜にタンパク抗原または特異抗体を共有結合させてリポソーム膜表面に抗原抗体複合体を形成させ、これに補体を作用させることにより膜攻撃複合体を形成し、該膜攻撃複合体によりリポソーム膜を破壊し、リポソーム内に封入されていたカルボキシフルオレセイン蛍光色素をリポソーム外へ放出させ、この放出されたカルボキシフルオレセイン蛍光色素の蛍光強度を測定することにより抗原量及び抗体量を測定することからなるリポソームを用いた免疫測定法において、カルボキシフルオレセイン蛍光色素の蛍光強度

比と共に透過率も同時に測定し、得られた両測定値に基づいてカルボキシフルオレセイン蛍光色素の濃度を決定することを特徴とするリポソームを用いた免疫測定法を提供する。

【００１１】

【作用】前記のように、本発明の方法によれば、カルボキシフルオレセイン蛍光色素の蛍光強度比と共に透過率も同時に測定する。カルボキシフルオレセイン蛍光色素の透過率は濃度に反比例する。すなわち、カルボキシフルオレセイン蛍光色素の濃度の増大につれて透過率は低下する。従って、既知のカルボキシフルオレセイン蛍光色素の濃度について蛍光強度比及び透過率の検量線を作成しておくことにより、得られた未知検体のカルボキシフルオレセイン蛍光色素の蛍光強度比及び透過率測定値を検量線に当てはめることにより検体内のカルボキシフルオレセイン蛍光色素濃度を短時間に確実に決定することができる。

【００１２】このようにして、カルボキシフルオレセイン蛍光色素濃度が短時間に確実に決定されるので、結果的に、抗原量及び抗体量も短時間に確実に推定することができ、臨床検査ばかりか様々な理化学基礎研究の推進にも大いに役立つことができる。

【００１３】なお、この明細書で使用される“蛍光強度比”という用語は、リポソームに封入されている全ＣＦ量に対する蛍光強度を意味する。また、”透過率”という用語は、照射光量に対するセル内の物質によって散乱されずに透過した光量の比率を意味する。

【００１４】

【実施例】以下、図面を参照しながら本発明を更に詳細に説明する。

【００１５】リポソームを用いた免疫測定法自体は公知である。例えば、“製薬工場”，Ｖｏｌ．７，Ｎｏ．５（１９８７）４２１頁～４２５頁，“生体の科学”，Ｖｏｌ．３８，Ｎｏ．５（１９８７年１０月）４９８頁～５００頁及び“臨床検査”，Ｖｏｌ．３４，Ｎｏ．７（１９９０年７月）８６８頁～８７１頁に詳細に説明されている。

【００１６】図１は本発明の方法を実施するのに使用できる光学測定系の一例の概要斜視図である。図示されているように、光源には波長４８８ｎｍのアルゴンレーザ１０を使用する。光源にはフラッシュキセノンなども使用できる。アルゴンレーザ１０から出たビームはビームエクスパンダーを通り、ＮＤフィルタ１４からアパーチャ１６を経て、検体の注入された石英製のセルキュベット１８に入射される。このセル１８を挟んで、光源と直線的位置関係に透過率測定手段を配置し、光源と直交するように蛍光強度測定手段を配置する。透過率測定手段は例えば、フィルタ（λ＝４８８ｎｍバンドパスフィルタ）２０と出力モニタ２２とから構成されている。一方、セル１８の検体中のカルボキシフルオレセイン蛍光色素（ＣＦ）から発せられた蛍光はピンホール３０を通り、コンデンサ（集光器）３２，３２により集光され、フィルタ（λ＝５１４ｎｍ）３４で蛍光成分以外の光を除去し、波長５１４ｎｍの蛍光を集光レンズ３６で集め、フィールドストップ３８を通してホトセンサ４０に入射させる。また、レーザ励起光の出力の変動は、蛍光強度比及び透過率の測定値にも影響する。このため、セル１８に入射される直前の励起光出力を測定するためのモニタ２３とこのモニタに励起光を入射させるためのハーフミラー２４が設けられている。このモニタ２３で検出された励起光強度を基準にする。このような構成の光学系を使用することにより検体の透過率と蛍光強度を同時に測定することができる。得られた蛍光強度の測定値を蛍光強度比の形に変換するには、リポソームを界面活性剤により破壊して得られた蛍光強度を１００％とし、リポソーム自身の蛍光強度を０％として各測定値を変換すればよい。

【００１７】図２は図１に示された光学測定系を用いて作成された抗原濃度（ｍｇ／ｍｌ）に対する蛍光強度比と４８８ｎｍの透過率の関係を示す標準検量線である。補体依存性膜損傷反応を利用する免疫測定法には、リリース法，競合法及びサンドイッチ法の３種類があるが、本発明の検量線はサンドイッチ法を用いて作成されている。サンドイッチ法とは、抗原をリポソーム表面中の抗体と、フリーの抗体（二次抗体）でサンドイッチ状態にし、補体を活性化することからなる。

【００１８】以下にサンドイッチ法における検量線作成の実例として、血清中に含まれ、炎症時にその含有量が増加することが知られているＣ－反応性タンパク質（以下「ＣＲＰ」という）を対象とした検量線の作成方法を示す。使用するリポソームは材質としてコレステロール，ジパルミトイルフォスファチジルコリンなど数種のリン脂質を混合して用いる。これにＣＦを封入して多重層リポソームを調製する。一次抗体は、抗ヒトＣＲＰマウスモノクローナル抗体を用い、結合基を作るために過ヨウ素酸で処理し、リポソームと結合させる。この一次抗体が結合したリポソームは、反応を安定させる為の吸収剤を添加した１０ミリモルのＴＥＳ緩衝液を用いて適当な濃度に希釈し、これをリポソーム試薬とする。また、この緩衝液はリポソームの自然崩壊を防ぐ機能も有している。検量線を作成するための各定点に用いる蛍光強度測定用の標準血清は、精製ＣＲＰ抗原を各定点の濃度になるように、上記緩衝液を用いて数段階に希釈したものを用いる。上記のものを準備した上で、検量線の求め方は、以下の要領により行う。リポソーム試薬を上記標準血清を一定量ずつ混合し、ヒト体温に近い３７℃で１時間反応させた。この混合液に二次抗体（例えば、抗ヒトＣＲＰウサギ抗体）溶液と、補体（例えば、モルモット血清）溶液を一定量添加し、更に１時間反応させて反応が安定化した後、各混合液の蛍光強度と透過率を測

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定し、図２に示されるような検量線を作成する。

【００１９】例えば、検体の透過率の測定値が７８％で蛍光強度比が４５％の場合、図２より、検体内のＣＲＰ濃度は０．１ｍｇ／ｍｌであると決定できる。従来のように蛍光強度比だけからＣＲＰ濃度を決定する場合、蛍光強度比が４５％に対応するＣＲＰ濃度は０．１ｍｇ／ｍｌの他に４ｍｇ／ｍｌの両方が得られ、何れが正しい濃度なのか即座には決定できない。透過率の曲線はＣＲＰ濃度の増大につれて指数関数的に減衰するので、所定の透過率に対応するＣＲＰ濃度の交点は一つしかない。従って、仮に検体の透過率の測定値と蛍光強度比の測定値に対応するＣＲＰ濃度が完全に一致しなくても、透過率の測定値に対応するＣＲＰ濃度に最も近い、蛍光強度比の測定値に対応するＣＲＰ濃度をもって検体のＣＲＰ濃度であると決定することもできる。

【００２０】このように、検体の透過率の測定値を同時に測定することにより、この透過率測定値に基づき、蛍光強度比測定値に対応する真正な抗原濃度を短時間に確実に決定することができる。従って、従来のＣＦの蛍光強度から濃度を推定する測定方法におけるような、濃度領域を限定したり、あるいは、測定対象を幾通りかに希釈して前後の特性から濃度推定を行う迂遠で冗長な操作は全て不要になる。

【００２１】以下、具体例により本発明の方法の実効性を例証する。

【００２２】実施例

補体依存性膜損傷反応を利用した抗体測定の具体例について説明する。試薬及び方法は前記と同様な試薬と方法を使用した。，検体としては、病院より供与されたＣＲＰ陽性血清について、吸収剤添加１０ミリモルＴＥＳ緩衝液により１００倍に希釈したものを検体として、その蛍光強度を測定することにより各検体中のＣＲＰ濃度を得た。従来から使用され、信頼性の高い抗原抗体反応時の沈降反応や凝集反応を光学的に検出するＴＩＡ法によっても上記と同じ検体のＣＲＰ測定を行い、両方法の相関を調べた。その結果、図４に示すような相関が得られ、相関計数も０．９８と良好で、本方法の信頼性が確認された。

【００２３】

【発明の効果】以上説明したように、本発明の方法によれば、カルボキシフルオレセイン蛍光色素の蛍光強度比と共に透過率も同時に測定する。既知のカルボキシフルオレセイン蛍光色素の濃度について蛍光強度比及び透過

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率の検量線を作成しておくことにより、得られた未知検体のカルボキシフルオレセイン蛍光色素の蛍光強度比及び透過率測定値を検量線に当てはめることにより検体内のカルボキシフルオレセイン蛍光色素濃度を短時間に確実に決定することができる。このようにして、カルボキシフルオレセイン蛍光色素濃度が短時間に確実に決定されるので、結果的に、抗原量及び抗体量も短時間に確実に推定することができ、臨床検査ばかりか様々な理化学基礎研究の推進にも大いに役立つことができる。

【００２４】このように、蛍光強度比と共に検体の透過率を同時に測定することにより、この透過率測定値に基づき、蛍光強度比測定値に対応する真正なＣＦ濃度を短時間に確実に決定することができる。従って、従来のＣＦの蛍光強度から濃度を推定する測定方法におけるような、濃度領域を限定したり、あるいは、測定対象を幾通りかに希釈して前後の特性から濃度推定を行う迂遠で冗長な操作は全て不要になる。

【図面の簡単な説明】

【図１】本発明の方法を実施するのに使用できる光学測定系の一例の概要斜視図である。

【図２】図１の装置を用いて作成された蛍光強度比と透過率との検量線を示す線図である。

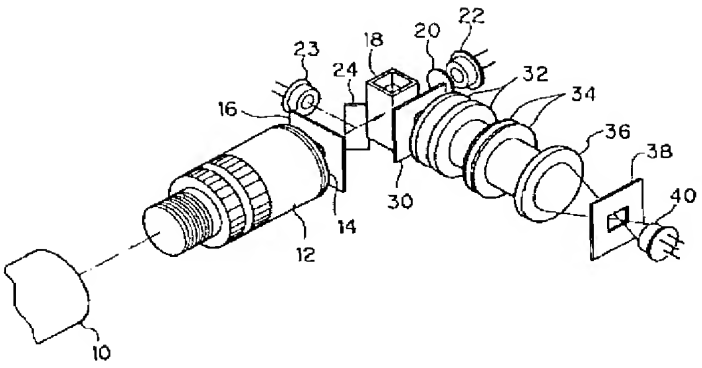
【図３】カルボキシフルオレセイン蛍光色素濃度と出力電圧値に換算した蛍光強度の関係を示す特性曲線の線図である。

【図４】本発明の方法により得られたＣＲＰ濃度測定値と従来のＴＩＡ法により得られたＣＲＰ濃度測定値の相関関係を示す特性図である。

【符号の説明】

１０　光源
１２　ビームエクスパンダー
１４　ＮＤフィルタ
１６　アパーチャ
１８　セル
２０　フィルタ
２２　出力モニタ
３０　ピンホール
３２　コンデンサ
３４　フィルタ
３６　集光レンズ
３８　フィールドストップ
４０　ホトセンサ

【図１】


10
12
14
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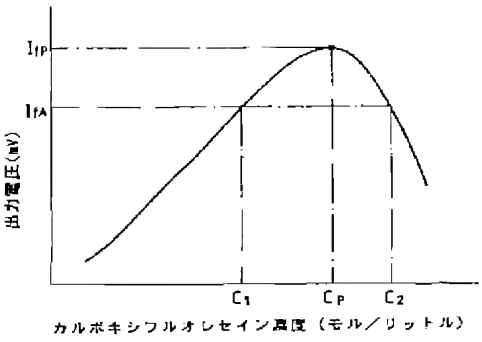
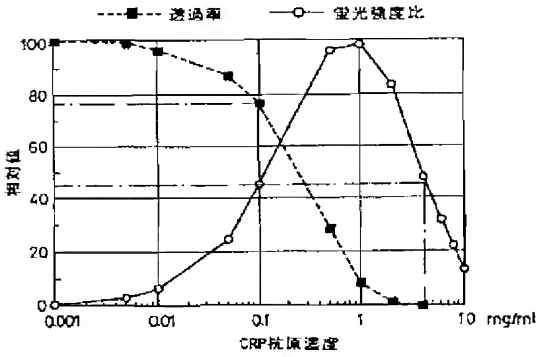
38
40

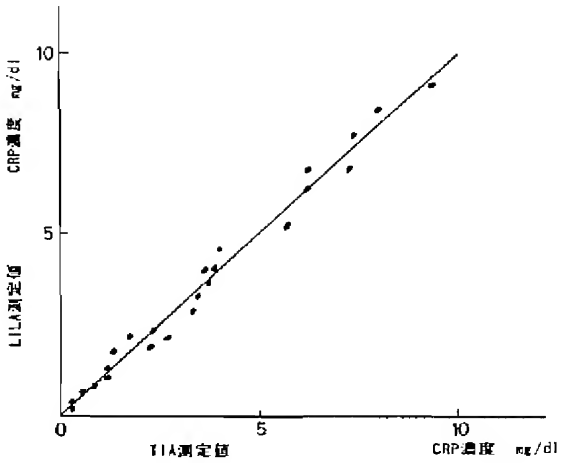
【図３】


IfP
IfA
出力電圧(mV)
C1
CP
C2
カルボキシフルオレセイン濃度（モル／リットル）

【図２】


透過率
蛍光強度比
100
80
60
40
20
0
相対値
0.001
0.01
0.1
1
10 mg/ml
CRP抗原濃度

【図４】


10
5
0
CRP濃度 mg/dl
LILA測定値
TIA測定値
CRP濃度 mg/dl

PATENT ABSTRACTS OF JAPAN

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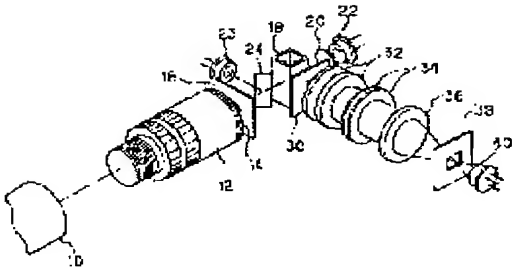
(72)Inventor : NEMOTO RYOJI
KASUGA RIKA

(54) IMMUNOASSAY METHOD USING LIPOSOME

(57)Abstract:
PURPOSE: To surely estimate the concentration of carboxyfluorescein (CF) in a short time by simultaneously measuring the fluorescence intensity ratio and transmittance of a CF fluorescent pigment and deciding the concentration of the pigment based on both measured values.
CONSTITUTION: An argon laser 10 is used as a light source and a laser beam from the laser 10 is made incident on a cell cuvet 18 containing an injected specimen through an ND filter 14 and aperture 16. A transmittance measuring means is positioned on the opposite side of the light source with respect to the cell 18 in a linear positional relation and a fluorescence intensity measuring means is positioned in a perpendicular positional relation with respect to the light source section. The fluorescence from a CF fluorescent pigment contained in the specimen is condensed by means of a condenser 32 through a pinhole 30 and made incident on a photosensor 40 after the light other than a fluorescent component is removed through a filter 34. The fluorescence of the pigment is measured on the basis of the intensity of excited light intensity detected by means of a monitor 23. When such an optical system constitution is used, the transmittance and fluorescence intensity of the specimen can be measured simultaneously. The conversion of the fluorescence intensity measured value into the fluorescence intensity ratio is performed by using the intensity of liposome destroyed by a surface-active agent and the intensity of the liposome itself.



LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

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CLAIMS

[Claim(s)]
[Claim 1] Enclose a carboxy fluorescein fluorochrome in liposome, carry out covalent bond of a protein antigen or the specific antibody to this liposome film, and an antigen antibody complex is made to form in a liposome film front face. By making complement act on this, form film attack complex and the liposome film is destroyed with this film attack complex. The carboxy fluorescein fluorochrome enclosed in liposome is made to emit out of liposome. In the immunoassay using the liposome which consists of measuring the amount of antigens, and the amount of antibodies by measuring the fluorescence intensity of this emitted carboxy fluorescein fluorochrome Immunoassay using the liposome characterized by also measuring transmission to coincidence and determining the concentration of a carboxy fluorescein fluorochrome based on both the obtained measured value with the fluorescence intensity ratio of a carboxy fluorescein fluorochrome.
[Claim 2] The approach of claim 1 which consists of determining the carboxy fluorescein fluorochrome concentration in a strange specimen by creating the calibration curve of a fluorescence intensity ratio and permeability beforehand about the carboxy fluorescein fluorochrome of known concentration, and applying the measured value of the fluorescence intensity ratio measured about the strange specimen, and permeability to said calibration curve.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the immunoassay which used liposome. Furthermore, this invention relates to the liposome immunoassay which can determine carboxy fluorescein fluorochrome concentration in a short time certainly at a detail in the immunoassay using the liposome which uses a carboxy fluorescein fluorochrome as a fluorescent substance.
[0002]
[Description of the Prior Art] Since the immunoassay by the antigen-antibody reaction is very excellent in respect of singularity and sensibility, it is widely used in many fields, such as physicochemistry research, medicine biology research, and a clinical laboratory test. In order to perform the microestimation of a specimen based on this antigen-antibody reaction, approaches, such as radioimmunoassay (RIA) by radioisotope and enzyme immunoassay (EIA) by the enzyme, have been developed.
[0003] However, although RIA is very excellent in respect of sensibility, since radioisotope is used as a marker, qualified person of the 1st sort person in charge of handling radiation is required, and if measurement is not in specific activity maintenance facilities, it cannot be carried out, but has troubles, such as needing the still more nearly special facility for the waste treatment of radioisotope.
[0004] Although a problem like RIA is not produced in EIA in order to use an enzyme as a marker, there is a trouble of having to separate an unreacted marker and measurement takes time and effort very much.
[0005] The immunoassay (LILA) which uses the liposome other than these approaches was proposed recently. Unlike conventional RIA or EIA, this LILA is the homogeneous system which does not need separation actuation of an unreacted marker, and has the advantage of being able to measure quickly moreover.
[0006] Liposome is an endoplasmic reticulum which consists of a lipid bilayer prepared by suspending the amphiphilic conjugated lipid (for example, lecithin, cholesterol, phosphatidic acid, etc.) which has both a hydrophilic group and a hydrophobic group in the same intramolecular in the buffer solution above fixed temperature. A bifunctional cross linking agent is used for this liposome film, and covalent bond of a protein antigen or the specific antibody is carried out to it. A carboxy fluorescein (CF) is enclosed as fluorescent labeling in liposome. CF is coloring matter of a fluorescein system, and water solubility is high and is easily enclosed in liposome. If a specific antibody or an antigen is made to react to this, an antigen antibody complex will be formed in a liposome film front face. If complement exists at this time, complement will be activated and film attack complex will be formed. It binds to the liposome film, the permeability of a lipid bilayer rises, and CF in liposome flows out into an external solvent. CF does not emit fluorescence by the self-quenching, when enclosed in liposome by high concentration, but if it flows out out of liposome and an external solvent dilutes, it will come to emit fluorescence. The amount of antigens and the amount of antibodies can be calculated by measuring this fluorescence intensity.
[0007]

[Problem(s) to be Solved by the Invention] It is CP, although, as for CF, fluorescence intensity and concentration maintain proportionality to a certain concentration (CP) according to the increment in mol concentration as shown in drawing 3 . The comparatively high-concentration field which exceeds shows the property that fluorescence concentration falls conversely. Therefore, the concentration of CF in fluorescence intensity IfA is C1. C2 Two kinds of values are acquired and it will become impossible to determine the concentration of what one in that of the right.
[0008] For this reason, whether the concentration field of CF being conventionally limited in the inspection approach of presuming concentration, from the fluorescence intensity of CF, and the measuring object were diluted to several kinds, and the roundabout and redundant approach of presuming concentration from the property of order was taken.
[0009] Therefore, the purpose of this invention is offering the new fluorescence detection approach the concentration of CF being presumed certainly in a short time, without taking a roundabout approach like before.
[0010]
[Means for Solving the Problem] In order to solve said technical problem, this invention encloses a carboxy fluorescein fluorochrome in liposome. Carry out covalent bond of a protein antigen or the specific antibody to this liposome film, and an antigen antibody complex is made to form in a liposome film front face. By making complement act on this, form film attack complex and the liposome film is destroyed with this film attack complex. The carboxy fluorescein fluorochrome enclosed in liposome is made to emit out of liposome. In the immunoassay using the liposome which consists of measuring the amount of antigens, and the amount of antibodies by measuring the fluorescence intensity of this emitted carboxy fluorescein fluorochrome The immunoassay using the liposome characterized by also measuring transmission to coincidence and determining the concentration of a carboxy fluorescein fluorochrome based on both the obtained measured value with the fluorescence intensity ratio of a carboxy fluorescein fluorochrome is offered.
[0011]
[Function] As mentioned above, according to the approach of this invention, permeability is also measured to coincidence with the fluorescence intensity ratio of a carboxy fluorescein fluorochrome. The permeability of a carboxy fluorescein fluorochrome is in inverse proportion to concentration. That is, permeability falls along with increase of the concentration of a carboxy fluorescein fluorochrome. Therefore, the carboxy fluorescein fluorochrome concentration in a specimen can be certainly determined in a short time by applying the fluorescence intensity ratio and transmissometry value of a carboxy fluorescein fluorochrome of a strange specimen which were acquired to a calibration curve by creating the calibration curve of a fluorescence intensity ratio and transmission about the concentration of a known carboxy fluorescein fluorochrome.
[0012] Thus, since carboxy fluorescein fluorochrome concentration is determined for a short time certainly, as a result, the amount of antigens and the amount of antibodies can also be presumed certainly in a short time, and can greatly be useful also to promotion of clinical laboratory tests or various physicochemistry fundamental researches.
[0013] In addition, the vocabulary the "fluorescence intensity ratio" used on these specifications means the fluorescence intensity to the total amount of CF enclosed with liposome. Moreover, the vocabulary "permeability" means the ratio of the quantity of light penetrated without being scattered about with the matter in the cel to the exposure quantity of light.
[0014]
[Example] Hereafter, this invention is further explained to a detail, referring to a drawing.
[0015] The immunoassay using liposome itself is well-known. For example, it is explained to a "pharmaceutical factory", Vol.7, No.5 (1987) 421 page -425 page, "a living body's science", Vol.38, No.5 (October, 1987) 498 page -500 page and a "clinical laboratory test", Vol.34, and No.7 (July, 1990) 868 page -871 page at the detail.
[0016] Drawing 1 is the outline perspective view of an example of the optical measurement system which can be used for enforcing the approach of this invention. The argon laser 10 with a wavelength of 488nm is used for the light source as illustrated. A flash plate xenon etc. can be used for the light source. The beam which came out of argon laser 10 passes along a beam

expander, and incidence is carried out to the cel cuvette 18 made from a quartz into which the specimen was poured through aperture 16 from ND filter 14. This cel 18 is pinched, a transmissometry means is arranged to the light source and linear physical relationship, and a fluorescence intensity measurement means is arranged so that it may intersect perpendicularly with the light source. The transmissometry means consists of a filter (lambda= 488nm band pass filter) 20 and an output monitor 22. On the other hand, it passes along a pinhole 30, and is condensed by capacitors (substage condenser) 32 and 32, and the fluorescence emitted from the carboxy fluorescein fluorochrome in the specimen of a cel 18 (CF) removes light other than a fluorescence component with a filter (lambda= 514nm) 34, collects fluorescence with a wavelength of 514nm with a condenser lens 36, and it is made it to carry out incidence to the phot sensor 40 through the field stop 38. Moreover, fluctuation of the output of laser excitation light also influences the measured value of a fluorescence intensity ratio and permeability. For this reason, the half mirror 24 for carrying out incidence of the excitation light to the monitor 23 and this monitor for measuring an excitation optical output just before incidence is carried out to a cel 18 is formed. It is based on the excitation light reinforcement detected with this monitor 23. The permeability and fluorescence intensity of a specimen can be measured to coincidence by using the optical system of such a configuration. What is necessary is just to change each measured value, making into 100% fluorescence intensity which destroyed liposome with the surfactant and was obtained, and using own fluorescence intensity of liposome as 0%, in order to change the measured value of the obtained fluorescence intensity into the form of a fluorescence intensity ratio.

[0017] Drawing 2 is a standard calibration curve which shows relation with a fluorescence intensity ratio [to the antigen concentration (mg/ml) created using the optical measurement system shown in drawing 1], and a permeability of 488nm. Although there are three kinds of immunoassay using a complement dependency film damage reaction, the releasing method, the competing method, and a sandwich technique, the calibration curve of this invention is created using the sandwich technique. An antigen is changed into a sandwiches condition by the antibody in a liposome front face, and the free antibody (second antibody), and it becomes a sandwich technique from activating complement.

[0018] The creation approach of the calibration curve for C-reactivity protein (henceforth "CRP") with which it is known that it will be contained in a blood serum as an example of the calibration-curve creation in a sandwich technique below, and the content will increase below at the time of inflammation is shown. The liposome to be used mixes and uses several sorts of phospholipid, such as cholesterol and dipalmitoyl phosphatidylcholine, as the quality of the material. CF is enclosed with this and multiplex layer liposome is prepared. Using an anti-Homo sapiens CRP mouse monoclonal antibody, a primary antibody is processed with periodic acid, in order to make a joint radical, and it is combined with liposome. The liposome which this primary antibody combined is diluted to suitable concentration using the TES buffer solution of 10 millimols which added the absorbent for stabilizing a reaction, and makes this a liposome reagent. Moreover, this buffer solution also has the function which prevents the spontaneous disintegration of liposome. in order to create a calibration curve -- each -- the reference serum for fluorescence intensity measurement used for the fixed point -- a purification CRP antigen -- each -- what was diluted to several steps using the above-mentioned buffer solution is used so that it may become the concentration of the fixed point. The following points perform how to search for a calibration curve, after preparing the above-mentioned thing. The above-mentioned reference serum was mixed the constant rate every, and the liposome reagent was made to react at 37 degrees C near Homo sapiens temperature for 1 hour. After carrying out constant-rate addition, making a second antibody (for example, anti-Homo sapiens CRP rabbit antibody) solution and a complement (for example, guinea pig serum) solution react to this mixed liquor for further 1 hour and a reaction's being stable, the fluorescence intensity and the permeability of each mixed liquor are measured, and a calibration curve as shown in drawing 2 is created.

[0019] For example, when the measured value of the permeability of a specimen is [a fluorescence intensity ratio] 45% at 78%, it can be determined from drawing 2 that the CRP concentration in a specimen is 0.1mg/ml. When determining CRP concentration only from a

fluorescence intensity ratio like before, 4mg [/ml] both are obtained by 0.1mg [/ml] others, and the CRP concentration corresponding to 45% in a fluorescence intensity ratio cannot determine immediately any are right concentration. Since the curve of permeability is exponentially decreased along with increase of CRP concentration, there is only one intersection of the CRP concentration corresponding to predetermined permeability. Therefore, even if the CRP concentration corresponding to the measured value of the permeability of a specimen and the measured value of a fluorescence intensity ratio is not completely in agreement, it can also determine to be the CRP concentration of a specimen with the CRP concentration corresponding to the measured value of a fluorescence intensity ratio nearest to the CRP concentration corresponding to the measured value of permeability.

[0020] Thus, based on this transmissometry value, the Shinsei antigen concentration corresponding to fluorescence intensity ratio measured value can be certainly determined in a short time by measuring the measured value of the permeability of a specimen to coincidence. Therefore, a concentration field [as / in the measuring method which presumes concentration from the fluorescence intensity of conventional CF] is limited, or all roundabout and redundant actuation of diluting the measuring object to several kinds and performing concentration presumption from the property of order becomes needlessness.

[0021] Hereafter, an example illustrates the effectiveness of the approach of this invention.

[0022] The example of antibody measurement in which the example complement dependency film damage reaction was used is explained. The reagent and the approach used the same reagent and same approach as the above. As a specimen, the CRP concentration in each specimen was obtained by measuring the fluorescence intensity about the CRP positivity blood serum supplied by the hospital by making into a specimen what was diluted 100 times with the absorbent addition 10 millimol TES buffer solution. TIA which is used from the former and detects optically the precipitation and agglutination reaction at the time of a reliable antigen-antibody reaction -- law -- CRP measurement of the same specimen as the above -- carrying out -- both -- correlation of a method was investigated. Consequently, correlation as shown in drawing 4 was acquired, the number of correlators was also as good as 0.98, and the dependability of this approach was checked.

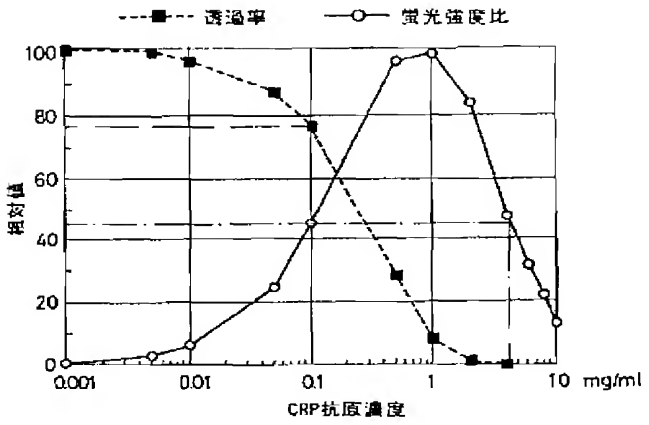
[0023]

[Effect of the Invention] As explained above, according to the approach of this invention, permeability is also measured to coincidence with the fluorescence intensity ratio of a carboxy fluorescein fluorochrome. By creating the calibration curve of a fluorescence intensity ratio and transmission about the concentration of a known carboxy fluorescein fluorochrome, the carboxy fluorescein fluorochrome concentration in a specimen can be certainly determined in a short time by applying the fluorescence intensity ratio and transmissometry value of a carboxy fluorescein fluorochrome of a strange specimen which were acquired to a calibration curve. Thus, since carboxy fluorescein fluorochrome concentration is determined for a short time certainly, as a result, the amount of antigens and the amount of antibodies can also be presumed certainly in a short time, and can greatly be useful also to promotion of clinical laboratory tests or various physicochemistry fundamental researches.

[0024] Thus, based on this transmissometry value, Shinsei CF concentration corresponding to fluorescence intensity ratio measured value can be certainly determined in a short time by measuring the permeability of a specimen to coincidence with a fluorescence intensity ratio. Therefore, a concentration field [as / in the measuring method which presumes concentration from the fluorescence intensity of conventional CF] is limited, or all roundabout and redundant actuation of diluting the measuring object to several kinds and performing concentration presumption from the property of order becomes needlessness.

[Translation done.]

Drawing selection [Representative drawing]



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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the outline perspective view of an example of the optical measurement system which can be used for enforcing the approach of this invention.
[Drawing 2] It is the diagram showing the calibration curve of the fluorescence intensity ratio and permeability which were created using the equipment of drawing 1 .
[Drawing 3] It is the diagram of a characteristic curve showing the relation between carboxy fluorescein fluorochrome concentration and the fluorescence intensity converted into the output voltage value.
[Drawing 4] the CRP density measurement value acquired by the approach of this invention, and conventional TIA -- it is the property Fig. showing the correlation of the CRP density measurement value acquired by law.
[Description of Notations]
10 Light Source
12 Beam Expander
14 ND Filter
16 Aperture
18 Cel
20 Filter
22 Output Monitor
30 Pinhole
32 Capacitor
34 Filter
36 Condenser Lens
38 Field Stop
40 Phot Sensor

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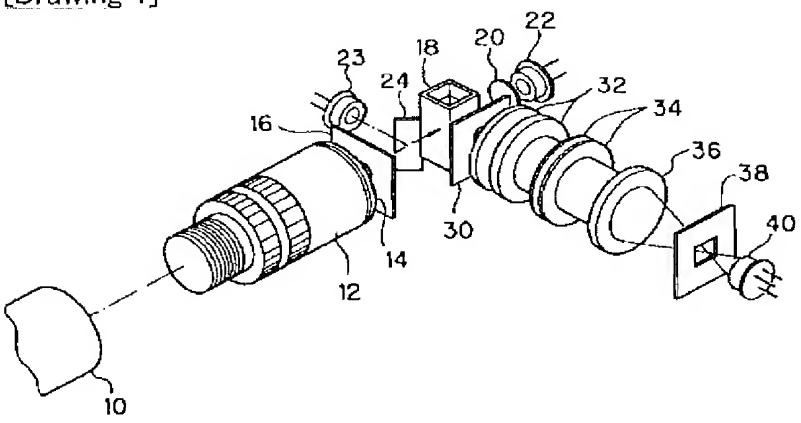
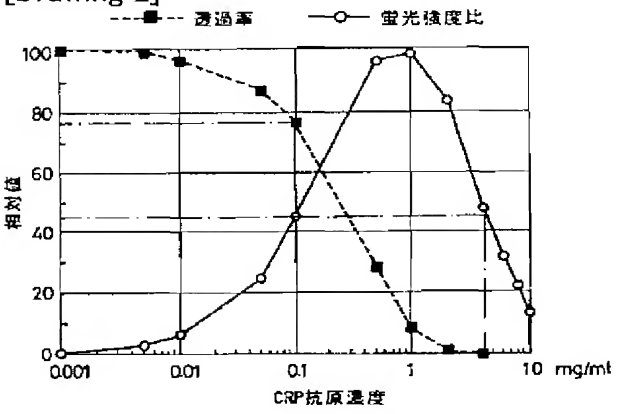
DRAWINGS

[Drawing 1]



[Drawing 2]



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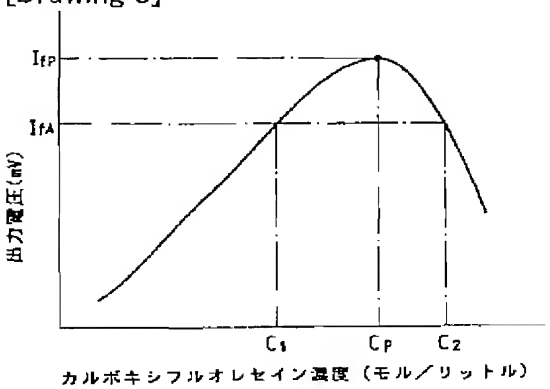

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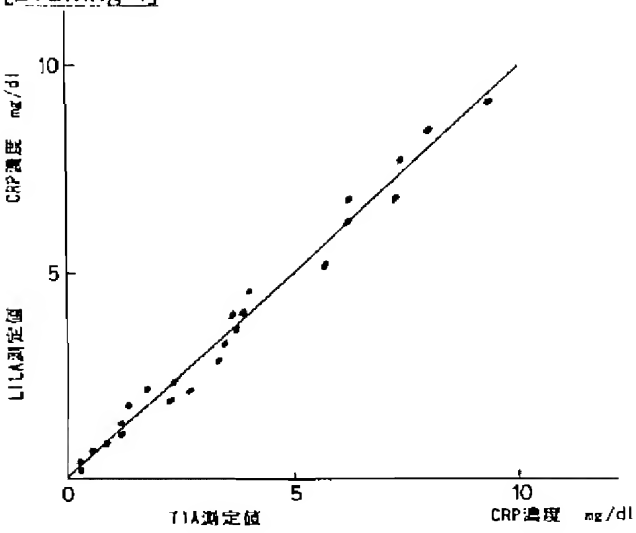

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